

WE CLAIM:

1. A method of detecting activation of a receptor on a cell comprising
contacting a luminescently labeled nucleotide with a specific binding partner,
5 wherein the extent of binding between the nucleotide and the specific binding partner
may be correlated to receptor activation, and

detecting luminescence polarization, wherein an increase in the level of
polarization indicates activation of the receptor.

10 2. The method of claim 1, wherein the step of detecting luminescence
polarization comprises evaluating a function selected from the group consisting of
polarization and anisotropy.

15 3. The method of claim 1, wherein the specific binding partner is selected
from the group consisting of antibodies and GTP-binding proteins.

4. The method of claim 1, wherein the nucleotide is selected from the group
consisting of cyclic AMP (cAMP), cyclic GMP (cGMP), and nonhydrolyzable GTP.

5. The method of claim 1 further comprising the step of
incubating whole cells or a cell lysate with a compound to determine the affect of
the compound on activation of the receptor.

5 6. The method of claim 1 further comprising the step of
correlating a detected level of luminescence polarization with activation of a
receptor selected from the group consisting of G-protein-coupled receptors.

7. A method of determining the effect of a candidate compound on activation
10 of an enzyme or an extracellular receptor comprising

assaying a sample containing an enzyme or an extracellular receptor in the
presence and in the absence of a candidate compound, wherein the assaying step
comprises contacting the sample with a luminescently labeled nucleotide,

15 detecting luminescence polarization indicating extent of binding between the
nucleotide and a specific binding partner, and

determining the effect of the candidate compound on enzyme or receptor
activation based on luminescence polarization, wherein an increase in the degree of
polarization in the presence as compared to the absence of a compound identifies the
compound as stimulating activation of the enzyme in a receptor, and a decrease in the
20 degree of polarization in the presence as compared to the absence of the compound
identifies the compound as inhibiting activation of the enzyme or receptor.

8. The method¹ of claim 7, wherein the step of detecting luminescence polarization comprises evaluating a function selected from the group consisting of polarization and anisotropy.

5 9. The method of claim 7, wherein the nucleotide is selected from the group consisting of cAMP, cGMP, and nonhydrolyzable GTP.

10. The method of claim 7, wherein the enzyme or receptor is selected from the group consisting of adenylyl cyclase, guanylyl cyclase, and G-protein-coupled receptors.

10 11. The method of claim 7 further comprising the step of providing the luminescently labeled nucleotide in a lysis buffer.

15 12. The method of claim 7, wherein the specific binding partner is selected from the group consisting of antibodies, G-protein-coupled receptors, serpentine receptors, and seven transmembrane-spanning domain receptors.

20 13. A nonpeptide luminescently labeled tracer comprising a luminophore coupled to a nonhydrolyzable form of guanosine triphosphate (GTP) for use in luminescence polarization assays.

14. The tracer of claim 13, wherein the nonhydrolyzable form of GTP is GTP(γ -S).

15. A method to measure the concentration of the activated form of a 5 7-transmembrane-spanning domain receptor which method comprises

contacting a sample containing the receptor with the labeled tracer of claim 3 under conditions wherein the labeled tracer binds the activated form of the receptor,

subjecting the sample to polarized light comprising a wavelength for excitation of the luminophore to effect emission of polarized light, and

10 determining the degree of polarization of the light emitted by the luminophore, whereby the degree of polarization of the emitted light is directly correlated with the concentration of the activated form of the receptor.

15. A method to determine the effect of a candidate compound on the activation of a 7-transmembrane-spanning domain receptor which method comprises

conducting the method of claim 5 in the presence and in the absence of the candidate compound, whereby an increase in the degree of polarization of the emitted light in the presence as compared to the absence of the compound identifies the compound as stimulating the activation of the receptor, and a decrease in the degree of 20 polarization of the emitted light in the presence as compared to the absence of the compound identifies the compound as inhibiting the activation of the receptor.

17. A nonpeptide luminescently labeled tracer comprising a cyclic nucleotide coupled to a luminophore for use in a luminescence polarization assay.

18. The tracer of claim 17, wherein the cyclic nucleotide is selected from the
5 group consisting of cAMP and cGMP.

19. The tracer of claim 17, wherein the cyclic nucleotide is coupled to the luminophore by a rigid coupling group.

10 20. The tracer of claim 17, wherein the rigid coupling group is a 1,2- or 1,4-diaminocyclohexyl coupling group.

21. The tracer of claim 17, wherein the tracer is selected from the group consisting of fluorescein-ITC-1,4-DACHsuccinimidyl cAMP, fluorescein-ITC-1,2-DACHsuccinimidyl cAMP, carboxyfluorescein-ITC-1,4-DACHsuccinimidyl cAMP, carboxyfluorescein-ITC-1,2-DACHsuccinimidyl cAMP, fluorescein-ITC-1,4-DACHsuccinimidyl cGMP, fluorescein-ITC-1,2-DACHsuccinimidyl cGMP, carboxyfluorescein-ITC-1,4-DACHsuccinimidyl cGMP, carboxyfluorescein-ITC-1,2-DACHsuccinimidyl cGMP.

22. A method of determining the concentration of a cyclic nucleotide, the method comprising

contacting a sample in which the concentration of the cyclic nucleotide is to be measured with the tracer of claim 17 and with the opposite member of a specific binding pair to the cyclic nucleotide,

illuminating the sample with polarized light, wherein the light is capable of inducing emission of polarized light from the luminophore,

measuring the extent of polarization of light emitted from the luminophore, and

correlating the extent of polarization of the emitted light with the concentration of the cyclic nucleotide.

23. The method of claim 22, wherein the cyclic nucleotide is selected from the group consisting of cAMP and cGMP.

15 24. The method of claim 22, wherein the opposite member of a specific binding pair is an immunological binding partner.

25. The method of claim 22, wherein the extent of polarization is measured using a function selected from the group consisting of polarization and anisotropy.

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26. The method of claim 22, wherein the extent of polarization of the emitted light is inversely correlated with the concentration of the cyclic nucleotide.

27. The method of claim 22 further comprising
determining the concentration of the cyclic nucleotide.

28. The method of claim 22, wherein the sample comprises whole cells.

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29. A method of identifying a compound as a modulator of a receptor or enzyme that generates a cyclic nucleotide, the method comprising
conducting the method of claim 22 in the presence and in the absence of the putative modulator, wherein an increase in the measured extent of polarization of the emitted light in the presence of the putative modulator in comparison with the measured extent in the absence of the putative modulator identifies the putative modulator as an inhibitor of the receptor or enzyme, and wherein a decrease in the measured extent of polarization in the presence of the putative modulator in comparison with the measured extent in the absence of the putative modulator identifies the putative modulator as an agonist of the receptor or enzyme.

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30. The method of claim 29, wherein the cyclic nucleotide is selected from the group consisting of cAMP and cGMP.

31. A kit for use in a luminescence assay for determining the concentration of a cyclic nucleotide or for identifying a compound as a modulator of a receptor or enzyme that generates a cyclic nucleotide, the kit comprising

an anti-cyclic nucleotide antibody, and

5 a tracer comprising a cyclic nucleotide coupled to a luminophore.

32. The kit of claim 31, wherein the cyclic nucleotide is selected from the group consisting of cAMP and cGMP.

10 33. The kit of claim 31 further comprising

a calibrator cyclic nucleotide.

34. The kit of claim 31 further comprising

a lysis buffer for lysing cells for cell-based assays.

15 35. A lysis buffer for use in a cell-based luminescence assay for determining the concentration of a cyclic nucleotide or for identifying a compound as a modulator of a receptor or enzyme that generates a cyclic nucleotide, wherein the buffer permits the assay to be performed without purification or processing steps, other than to add 20 polarization reagents, incubate, and read.